# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference<br>033028woMest | **FOR FURTHER ACTION** | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| International application No.<br>PCT/EP 03/14661 | International filing date *(day/month/year)*<br>19.12.2003 | Priority date *(day/month/year)*<br>20.12.2002 |
| International Patent Classification (IPC) or both national classification and IPC<br>C12Q1/68 | | |
| Applicant<br>EVOTEC OAI AG et al. | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 15.06.2004 | 16.07.2004 |
| Name and mailing address of the international preliminary examining authority:<br>European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Authorized Officer<br>Bulcao de Melo Barre<br>Telephone No. +49 89 2399-8972 |

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/14661

## I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

**Description, Pages**

1-37 as originally filed

**Claims, Numbers**

1-40 as originally filed

**Drawings, Sheets**

1/12-12/12 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   *(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

## V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 1-40 |
| | No: | Claims | |
| Inventive step (IS) | Yes: | Claims | 1-40 |
| | No: | Claims | |
| Industrial applicability (IA) | Yes: | Claims | 1-40 |
| | No: | Claims | |

2. Citations and explanations

   **see separate sheet**

1. Reference is made to the following documents:

   **D1**: WO 90/01564

2. Novelty (**Article 33(2) PCT**)

   The subject-matter of the present application does not appear to be disclosed in the prior art as defined in the regulations (**Rule 64 (1)-(3) PCT**).
   Therefore, in view of such prior art the subject-matter of the present application (**claims 1-40**) has to be regarded as being new (**Article 33(2) PCT**).

3. Inventive Step (**Article 33(3) PCT**)

   The **closest prior art** to evaluate the inventiveness of the subject-matter of the present application is **D1**.
   Document **D1** discloses a method for detecting target nucleic acids comprising contacting the target nucleic acids (analyte) with a solid support having different nucleic acids covalently bound thereto (capture oligos). Said capture nucleic acids are complementary to the target sequences, which are preferably RNA sequences. The method further comprises contacting the above complex (analyte-capture oligos on the solid support) with a detectable nucleic acid probe (detection oligos), which is complementary to sequences of the target nucleic acid that are different from the sequences to which the immobilized nucleic acid are complementary.
   Both capture and detection oligos are labelled by e.g. fluorescent or chemiluminescent compounds. The solid support is e.g. a bead and the capture oligos bound thereto are biotinylated.
   (See Abstract; page 5, line 9 - page 9, line 12; page 16, line 30 - page 17, line 15; page 21, line 6 - page 26, line 21; page 31, line 10 - page 35, line 29; examples, claims and figure 1)

   Starting from **D1** the underlying **technical problem** to be solved by the present application is the provision of an alternative method for detecting an analyte in a sample.

   The **solution** provided by the Applicant to solve the above technical problem is a

method as defined in D1, wherein the detection of the detection probes/oligonucleotides (DO) is conducted in the presence of quenching probes and/or the solid support is labelled with a second reporter different from the first reporter labelling the DO.

None of the available prior art documents suggest the above solution to improve the sensitivity of the method of D1.
The method of the present invention shows significant improved sensitivity due to the additional steps of adding quenching probes/oligonucleotides and unspecific labelling of the solid support with a second reporter.
The quenching probes/oligonucleotides bind to free surplus DO (i.e. not binding the analyte) thereby minimizing, at least partially, an emission of the first reporter of said surplus DO. The quenching probes/oligonucleotides therefore advantageously reduce the background signal caused by unbound DO.
The labelling of the solid support with a second reporter different from the first reporter labelling the DO allows to generate a mask, from imaging the sample at an emission wavelength of the second reporter (reference image), and apply this mask to an image of the sample at an emission wavelength of the first reporter (sample image). The recognition of the solid support using a reporter labelling the solid support (second reporter) is advantageous because otherwise contaminations of the sample or sufficiently large aggregates of DO would be recognized erroneously as signal stemming from the first reporter of the DO-analyte-complex.

Therefore, the subject-matter of the present application (**claims 1-40**) is considered to involve an inventive step (**Article 33(3) PCT**).

4. Industrial Applicability (**Article 33(4) PCT**)

The subject-matter of the present application (**claims 1-40**) is susceptible of industrial applicability as defined in **Article 33 (4) PCT**.

5. Further Observations

5.1. The Applicant is informed that expressions like "in particular" (**claims 4, 6, 12, 13 and 36**), "preferably" (**claim 26**), and "more preferably", "even more preferably" and "most preferably" (**claims 8 and 18**) have no limiting effect on the scope of the claims, that is to say, the features following any such expressions are to be regarded as entirely optional (see the **Guidelines for Preliminary Examination PCT, CIII 4.6**).

5.2. Contrary to the requirements of **Rule 5.1(a)(ii) PCT**, the relevant background art disclosed in document **D1** is not mentioned in the description, nor is this document identified therein.